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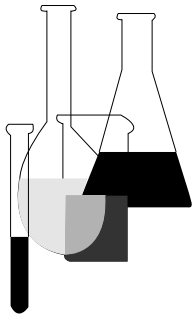
Health Effects Test Guidelines

OPPTS 870.5915
In Vivo Sister Chromatid Exchange Assay



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5915 In vivo sister chromatid exchange assay.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5915 In vivo sister chromatid exchange assay and OPP 84–2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** The sister chromatid exchange (SCE) assay detects the ability of a chemical to enhance the exchange of DNA between two sister chromatids of a duplicating chromosome. The test may be performed *in vitro* using cultured mammalian cells or *in vivo* using nonmammalian or mammalian tissues. The most commonly used assays employ bone marrow or lymphocytes from mammalian species such as mice, rats, or hamsters. Human lymphocytes may also be used.

(c) **Definition.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definition also applies to this test guideline.

Sister chromatid exchanges are reciprocal interchanges of the two chromatid arms within a single chromosome. These exchanges are visualized during the metaphase portion of the cell cycle and presumably require enzymatic incision, translocation and ligation of at least two DNA helices.

(d) **Test method**—(1) **Principle.** Animals are exposed to test substance by appropriate routes followed by administration of bromodeoxyuridine (BrdU). A spindle inhibitor (e.g., colchicine or Colcemid®) is administered prior to sacrifice. After sacrifice, tissue is obtained and metaphase preparations made, stained, and scored for SCE.

(2) **Description.** The method described here employs bone marrow of laboratory rodents exposed to test chemicals. After treatment with test chemical, animals are further treated with BrdU and, prior to sacrifice, with a spindle inhibitor (e.g., colchicine or Colcemid®) to arrest cells in c-metaphase. After sacrifice, chromosome preparations from bone marrow cells are made, stained, and scored for SCE.

(3) **Animal selection**—(i) **Species and strain.** Any appropriate mammalian species may be used. Examples of commonly used rodent species include mice, rats, and hamsters.

(ii) **Age.** Healthy, young adult animals should be used.

(iii) **Number and sex.** At least five female and five male animals per experimental and control group should be used. The use of a single sex or different number of animals should be justified.

(iv) **Assignment to groups.** Animals should be randomized and assigned to treatment and control groups.

(4) **Control groups**—(i) **Concurrent controls.** Current positive and negative (vehicle) controls should be included in the assay.

(ii) **Positive controls.** A compound know to produce SCE *in vivo* should be employed as the positive control.

(5) **Test chemicals**—(i) **Vehicle.** When possible, test chemicals should be dissolved in isotonic saline or distilled water. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test compound nor produce toxic effects. Fresh preparations of the test compound should be employed.

(ii) **Dose levels.** For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose or that producing some indication of toxicity as evidenced by animal morbidity (including death) or target cell toxicity. The LD_{50} is a suitable guide. Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

(iii) **Route of administration.** The usual routes of administration are IP or oral. Other routes may be appropriate.

(iv) **Treatment schedule.** In general, test substances should be administered only once. However, based upon toxicological information a repeated treatment schedule may be employed.

(e) **Test performance**—(1) **Treatment.** Animals should be treated with test chemical followed by administration of BrdU. BrdU may be administered by multiple IP injections, by continuous tail vein infusion or by subcutaneous implantation of tablets. Animals should be treated with a spindle inhibitor (e.g., colchicine or Colcemid®) 2 hours prior to sacrifice. After sacrifice, bone marrow should be extracted and slides made and prepared for SCE evaluation.

(2) **Staining method.** Staining of slides to reveal SCEs can be performed according to any of several protocols. However, the fluorescence plus Giemsa method is recommended.

(3) **Number of cells scored.** The number of cells to be analyzed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity and the power chosen for the test. Slides should be coded before microscopic analysis.

(f) **Data and report**—(1) **Treatment of results.** Data should be presented in tabular form, providing scores for both the number of SCE for each metaphase and the number of SCE per chromosome for each metaphase. Differences among animals within each group should be considered before making comparisons between treated and control groups.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of SCE. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of SCE or a statistically significant and reproducible positive response at any one of the test points is considered not to induce rearrangements of DNA segments in this system.

(iii) Both biological and statistical significance should be considered in the evaluation.

(4) **Test evaluation.** (i) Positive results in the *in vivo* SCE assayindicate that under the test conditions the test substance induces reciprocal interchanges in the bone marrow of the test species.

(ii) Negative results indicate that under the test conditions the test substance does not induce reciprocal interchanges in the bone marrow of the test species.

(5) **Test report.** In addition to the reporting recommendations as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Species, strain, age, weight, number, and sex of animals in each treatment and control group.

(ii) Test chemical vehicle, dose level used, rationale for dose selection, toxicity data, negative and positive controls.

(iii) Route and schedule of administration of both test chemical and BrdU.

(iv) Identity of spindle inhibitor, its concentration and duration of treatment.

(v) Time of sacrifice after administration of BrdU.

(vi) Details of the protocol used for slide preparation.

(vii) Criteria for scoring SCE.

(viii) Dose-response relationship, if applicable.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Allen, J.W. et al. Bromodeoxyuridine tablet methodology for in vivo studies of DNA synthesis. *Somatic Cell Genetics* 4:393–405 (1978).

(2) Allen, J.W. et al. Simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromodeoxyuridine tablets. *Cytogenetics Cell Genetics* 18:231–237 (1977).

(3) Latt, S.A. et al. Sister chromatid exchanges: A report of the U.S. EPA Gene-Tox Program. *Mutation Research* 87:17–62 (1981).